Test Methods for Measuring *E. coli* in Wastewater

Introduction

The EPA has approved three approaches in 40 CFR 136 for quantifying *E. coli in wastewater*: membrane filtration, multiple tube/multiple well, and multiple tube fermentation. These three approaches are also approved by the Wisconsin DNR for *E. coli* monitoring in wastewater and are listed in Ch. NR 219, Wisc. Admin. Code.

A description of each of these approaches is provided below and a summary of the advantages and disadvantages of each is included in the table on page 3.

Recreation Water Quality Criteria for Bacteria

Recreation water quality criteria for bacteria protect people from exposure to bacteria that are present in water contaminated by human fecal matter.

Because pathogens can be difficult to measure directly, a pathogen indicator is used to signal the potential for illness caused by fecal contamination. The U.S. EPA recommends that *E. coli* or enterococci be used as the pathogen indicator.

Membrane Filtration

In the membrane filtration approach, a water sample is filtered through a membrane. The membrane is then placed on culture media that is selective for *E. coli*. Because the bacteria are retained on the surface of the filter, they grow on the media and develop into a visible colony.

The number of colonies that are formed are counted and reported as the colony forming units (CFUs).

mColiblue-24® by Hach Company is a commercially available culture media that can be used to quantify *E. coli* via the membrane filtration approach.
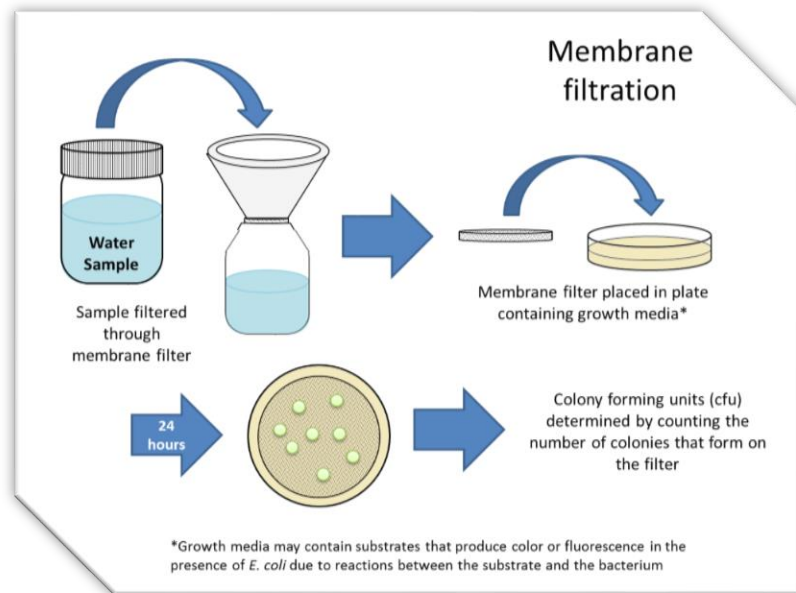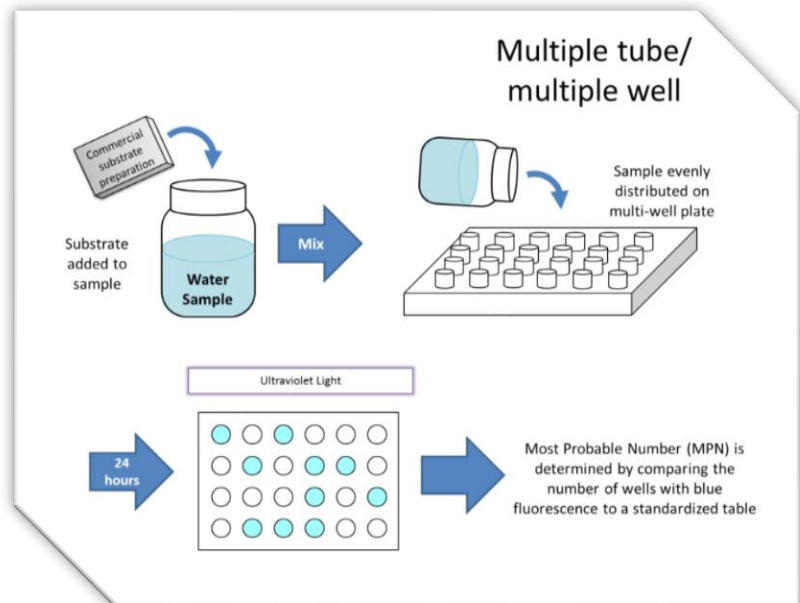
Multiple Tube/Multiple Well

In the multiple tube/multiple well approach, a water sample is mixed with a commercial reagent containing methylumbelliferyl-β-glucuronide (MUG). *E. coli* enzymatically cleaves MUG forming a fluorescent product. Samples are distributed into a multi-well plate. After incubating for 24 hours, the MPN is estimated from the number of wells that are positive for the presence of bacteria growth using a standardized table. The MPN is a statistical estimate of the mean bacteria density.

Colilert®and Colilert-18® by IDEXX Technologies are commercially available kits that can be used to quantify *E. coli* via the multiple tube/multiple well approach.

Multiple Tube Fermentation

The multiple tube fermentation approach is a two-step process. First, a water sample is added to test tubes containing bacteria growth media and incubated for 24-48 hrs. Tubes that are positive for the production of acid and/or gas are then added into a series of tubes with media containing MUG. After 24 hours, the tubes are examined for fluorescence.
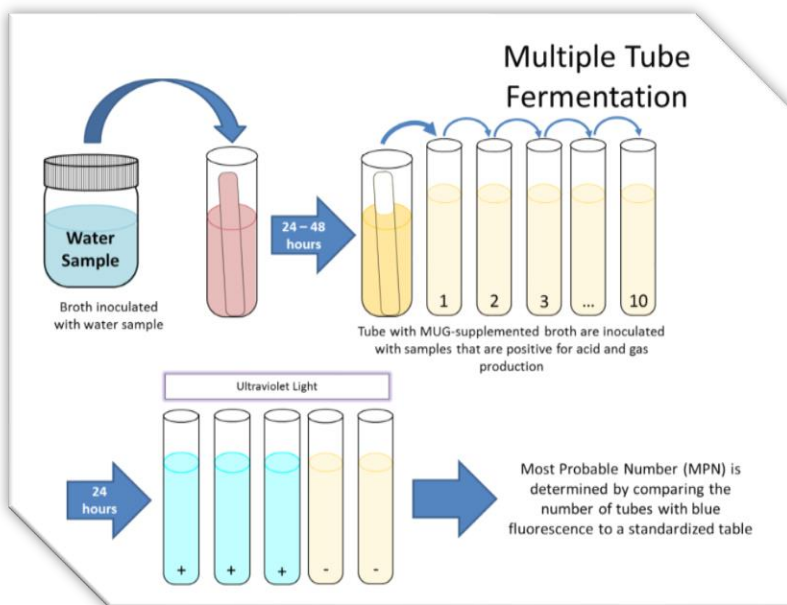
The bacteria level is reported as the most probable number (MPN). The MPN is estimated from the number of tubes that are positive for the presence of bacteria growth using a standardized table.

This approach is not used frequently as the precision is low unless a large number of samples are collected and it is more labor and time intensive than the other approaches.

EPA and Wisconsin DNR Approved Analytical Approaches for Quantifying *E. coli*				
Analytical Approach	**Standardized Test Method**	**Commercial Technology**	**Advantages**	**Disadvantages**
Membrane filtration: **Single-step or Two-step**	EPA 1603 SM 9222B-2015 SM 9222I-2015	mColiBlue-24® N/A	• Readily available • Used to establish EPA's *E. coli* criteria[3] • Results can be compared directly to fecal coliform results • Media less costly	• Labor and material intensive • Require high degree of technical skill to evaluate results • Additional analysis may be needed for samples with high turbidity, high levels of noncoliform bacteria, or organisms stressed by chlorine
Multiple tube/ multiple well	SM 9223-B-2016 AOAC 991.15	Colilert®[1] Colilert-18®[1]	• Commercially available • Standardized media and procedure • Less labor, material, and time intensive • Requires minimal technical skill to evaluate results	• May yield higher values than membrane filtration methods[2] • Reagent more costly • Requires specialized equipment
Multiple tube fermentation	SM 9221B.3–2014 SM 9221F–2014	N/A	• One of the first approved methods for quantifying *E. coli*	• Not commonly used • Labor and time intensive • May underestimate bacterial density

1. The advantages listed are specific to the Colilert® technologies.
2. Potential causes of discrepancies may include: (1) a greater-than-average false-positive rate with Colilert®; (2) a high number of false negatives with membrane filtration; (3) the ability for Colilert® to detect injured and viable but non-culturable bacterial cells while these cells cannot be detected via membrane filtration.
3. Membrane filtration was used to quantify *E. coli* in EPA's 1986 Ambient Water Quality Criteria for Bacteria. The EPA used the 1986 *E. coli* data in their 2012 Recreational Water Quality Criteria because new *E. coli* data was not collected as part of the epidemiological studies.

SM = Standard Methods for the Analysis of Water and Wastewater
AOAC = Association of Analytical Chemists

Additional Resources

- Analytical Test Methods and Procedures. *Wisconsin Administrative Code,* Chapter NR 219, 2020. https://docs.legis.wisconsin.gov/code/admin_code/nr/200/219.pdf
- Bain RE, *et al*. 2015. Evaluation of an inexpensive growth medium for direct detection of Escherichia coli in temperate and sub-tropical waters. PLoS One 10(10): e0140997.
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- Edge, T. A. and Boehm, A. B. (2011). Classical and molecular methods to measure fecal bacteria. In Sadowsky and R. L. Whitman (Eds.), *The Fecal Bacteria* (241-273). Washington, DC: American Society for Microbiology.
- Hach Company. *m-ColiBlue24® Broth, Plastic Ampules, PK/50*. http://www.hach.com/m-coliblue24-broth-plastic-ampules-pk-50/product?id=7640249626&callback=pf
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- IDEXX Laboratories. *Colilert®*. https://www.idexx.com/water/products/colilert.html
- Guidelines Establishing Test Procedures for the Analysis of Pollutants. *Code of Federal Regulations*, 40 "CFR" 136, 2014. http://www.ecfr.gov/cgi-bin/text-idx?SID=b104ff3b9795753b09a5aac5af6eaf95&mc=true&node=pt40.25.136&rgn=div5
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- Olstadt, J. et al. A comparison of ten USEPA approved total coliform/*E. coli* tests. *Journal of Water and Health*, 2007, 267-282.
- State of Oregon – Department of Environmental Quality (2003) Memorandum: *E. coli* methods and holding times. http://cwwuc.org/reference/prehearingstmt/Exhibit5.pdf
- State of Washington – Department of Ecology (2011) *Alternative bacteria source identification using Colilert®/Quanti-Tray 2000 test methods in irrigated agricultural watersheds.* http://www.svid.org/images/November%2017%20Final%20%20Report%20for%20Contract.pdf
- United States Environmental Protection Agency (1986) Ambient Water Quality Criteria for Bacteria. https://www.regulations.gov/contentStreamer?documentId=EPA-HQ-OW-2007-0808-0001&disposition=attachment&contentType=pdf
- United States Environmental Protection Agency (2012) Recreational Water Quality Criteria https://www.epa.gov/sites/production/files/2015-10/documents/rwqc2012.pdf

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